

Judy R. Freshour, Sharon E. Chase and Karen L. Vikstrom

Am J Physiol Heart Circ Physiol 283:1997-2003, 2002. First published Jul 18, 2002;

doi:10.1152/ajpheart.01054.2001

You might find this additional information useful...

This article cites 49 articles, 30 of which you can access free at:

<http://ajpheart.physiology.org/cgi/content/full/283/5/H1997#BIBL>

This article has been cited by 6 other HighWire hosted articles, the first 5 are:

NF- κ B activation is required for adaptive cardiac hypertrophy

L. Zelarayan, A. Renger, C. Noack, M.-P. Zafiriou, C. Gehrke, R. van der Nagel, R. Dietz, L. de Windt and M. W. Bergmann

Cardiovasc Res, December 1, 2009; 84 (3): 416-424.

[Abstract] [Full Text] [PDF]

Physiology of local Renin-Angiotensin systems.

M. Paul, A. Poyan Mehr and R. Kreutz

Physiol Rev, July 1, 2006; 86 (3): 747-803.

[Abstract] [Full Text] [PDF]

Regulation of components of the brain and cardiac renin-angiotensin systems by 17beta-estradiol after myocardial infarction in female rats

S. A. Dean, J. Tan, R. White, E. R. O'Brien and F. H. H. Leenen

Am J Physiol Regulatory Integrative Comp Physiol, July 1, 2006; 291 (1): R155-R162.

[Abstract] [Full Text] [PDF]

Mouse model carrying H222P-Lmna mutation develops muscular dystrophy and dilated cardiomyopathy similar to human striated muscle laminopathies

T. Arimura, A. Helbling-Leclerc, C. Massart, S. Varnous, F. Niel, E. Lacene, Y. Fromes, M. Toussaint, A.-M. Mura, D. I. Keller, H. Amthor, R. Isnard, M. Malissen, K. Schwartz and G. Bonne

Hum. Mol. Genet., January 1, 2005; 14 (1): 155-169.

[Abstract] [Full Text] [PDF]

Gender modulates cardiac phenotype development in genetically modified mice

X.-J. Du

Cardiovasc Res, August 15, 2004; 63 (3): 510-519.

[Abstract] [Full Text] [PDF]

Updated information and services including high-resolution figures, can be found at:

<http://ajpheart.physiology.org/cgi/content/full/283/5/H1997>

Additional material and information about *AJP - Heart and Circulatory Physiology* can be found at:

<http://www.the-aps.org/publications/ajpheart>

This information is current as of November 30, 2009 .

Gender differences in cardiac ACE expression are normalized in androgen-deprived male mice

JUDY R. FRESHOUR, SHARON E. CHASE, AND KAREN L. VIKSTROM

*Department of Pharmacology, State University of New York
Upstate Medical University, Syracuse, New York 13210*

Received 3 December 2001; accepted in final form 12 July 2002

Freshour, Judy R., Sharon E. Chase, and Karen L. Vikstrom. Gender differences in cardiac ACE expression are normalized in androgen-deprived male mice. *Am J Physiol Heart Circ Physiol* 283: H1997–H2003, 2002. First published July 18, 2002; 10.1152/ajpheart.01054.2001.—Gender differences have been described in the response of the cardiovascular system to a number of stimuli, including ventricular remodeling in response to pressure overload, but the molecular basis for these differences remains unclear. Because gender differences in the cardiac expression of angiotensin-converting enzyme (ACE) could contribute to differences in myocardial remodeling, we examined myocardial ACE expression in age-matched male and female mice. Ventricular ACE was more abundant in male than female mice at both mRNA and protein levels. These differences became apparent once the mice reached sexual maturity and became more pronounced with increasing age. The influence of mouse gonadal status on ventricular ACE expression was also examined. Oophorectomy slightly increased ACE levels in female mice, whereas ventricular ACE levels were substantially decreased in androgen-deprived males. The antithetical changes in ventricular ACE abundance seen in agonadal male and female mice suggest that testosterone as well as estrogen may play a role in regulating ACE expression in the heart.

angiotensin; angiotensin-converting enzyme; gene expression; estrogen; testosterone

THE LOWER INCIDENCE of cardiovascular disease in premenopausal women compared with men and postmenopausal women has led to the hypothesis that estrogens are cardioprotective. However, the Heart and Estrogen/Progestin Replacement Study failed to demonstrate decreased cardiovascular risk in postmenopausal women receiving estrogen replacement therapy (21). Moreover, epidemiological evidence suggests that the male gender is a risk factor for cardiovascular disease, but it is unclear what role androgens play in modulating cardiac gene expression. Consideration of these facts raises the following questions: 1) do both estrogens and androgens contribute to gender differences in the heart, and 2) what influences do these hormones have on the molecular phenotype of the heart in pathological states?

After menopause, the incidence of cardiovascular disease increases in women as estrogen levels decrease. Because the postmenopausal drop in estrogen levels may contribute to the increased risk of cardiovascular disease, significant efforts have been spent identifying estrogen-responsive behaviors of the heart and vasculature (for reviews, see Refs. 16, 27, and 32). Cardiovascular adaptations that have been demonstrated to be influenced by circulating estrogen levels include the vascular proliferative response to injury (14) and the development of endothelial dysfunction (44) and cardiac hypertrophy (37, 39). The influence of estrogens on these cardiovascular adaptations may reflect underlying transcriptional changes mediated by ligand-bound estrogen receptors of genes such as endothelial nitric oxide synthase (29) or cyclooxygenase (22). Alternatively, nontranscriptional effects of estrogen may be involved. It is clear that estrogen levels have a profound impact on cardiovascular function in females, but can we account for the lower incidence of cardiovascular disease in premenopausal women through estrogenic effects only? To answer this question requires a complete accounting of gender differences in cardiovascular biology and the role of sex hormones in modulating those differences.

Numerous gender-specific differences have been described in the cardiovascular system of humans and experimental animals. For example, several measures of cardiac performance (coronary flow, end-diastolic volume, stroke work, ejection fraction, and fractional shortening) are greater in male rats than in female rats when hearts of equivalent size are compared (35). Gender differences also have been demonstrated in the response of rats to catecholamines, hypoxia, physical training, or increased afterload (3, 28, 33, 34, 43, 46). A gender difference in cardiomyocyte size also has been described with larger cardiomyocytes found in adult male rats than in females (2). At the molecular level, it is likely that gender differences in cardiac physiology and pathophysiology reflect the sum of differential expression/activity of numerous molecules that impact cardiac function. The twofold greater phospho-Akt (protein kinase B) seen in female mouse hearts com-

Address for reprint requests and other correspondence: K. L. Vikstrom, Dept. of Pharmacology, SUNY Upstate Medical Univ., 750 East Adams St., Syracuse, NY 13210 (E-mail: vikstrok@upstate.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

pared with males (8) is one gender difference that has recently been identified.

It has been proposed that increased angiotensin-converting enzyme (ACE) abundance in the hypertrophied and failing heart may contribute to the local generation of angiotensin II and impact cardiac remodeling through local paracrine or autocrine effects (1, 45). In this report, we demonstrate a gender difference in the expression of ACE in the murine heart that suggests that differences in the cardiac expression of ACE could contribute to the gender differences in cardiac adaptation to stimuli such as physical training, hypoxia, or remodeling in response to pressure overload.

METHODS

Animal husbandry. Mice were maintained on a 10:14-h light-dark cycle in microisolator cages with free access to water and food. Swiss-Webster and C57/BL6 mice were purchased from Taconic Farms and Jackson Laboratories, respectively. A previously described transgenic mouse model for hypertrophic cardiomyopathy was also used for this study. These animals express a mutant α -myosin heavy chain with expression driven by a rat α -heavy chain promoter (for details, see Ref. 41). The transgenic line was maintained by crossing transgenic males with C57BL/6J females (Jackson Laboratories). Pups were genotyped by PCR, and non-transgenic littermates were used for age-matched controls.

Surgical procedures. Atropine sulfate (0.08 mg/100 g ip) was administered to 6- to 8-wk-old mice, and the animals were then anesthetized with ketamine (42 mg/100 g body wt ip) and xylazine (2 mg/100 g body wt ip). In males, the testes were exposed via bilateral midscrotal incisions, the vas deferens was ligated with 5-0 coated vicryl, and the caudal epididymis and testes were removed. In females, a small transverse incision was made at the approximate level of the last rib, another incision was made through the body wall, and the fat pad was pulled through the opening to expose the ovaries. The fallopian tubes were ligated, and the ovaries were removed. Analgesia was administered postoperatively (0.05–0.1 mg/kg sc buprenorphine) and as needed for 2–3 days after the surgery. Animals were euthanized, and tissues were collected 5 mo after the surgery. All animal protocols were approved by the Committee for the Humane Use of Animals at the State University of New York Upstate Medical University.

RNA analysis. Animals were euthanized humanely, and their hearts were excised. After being rinsed in Krebs-Henseleit buffer (118 mM NaCl, 4.7 mM KCl, 2.52 mM CaCl₂, 1.64 mM MgSO₄, 25 mM NaHCO₃, 1.18 mM KH₂PO₄, 5.55 mM glucose, and 2.0 mM sodium pyruvate), the right ventricle (RV) was dissected free from the left ventricle (LV) and septum under a stereomicroscope. The tissues were flash frozen in liquid nitrogen and stored at -70°C . Total RNA was isolated using the guanidinium-acid phenol method (10), and ACE mRNA levels were then measured using an RNase protection assay. A riboprobe specific for the somatic isoform of mouse ACE was generated by amplifying a 272-bp cDNA (gb:J03940, nucleotides 69–341) by RT-PCR. The PCR product was subcloned into the pCR-Script vector (Stratagene; LaJolla, CA), and the sequence was verified. The mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA clone (gb:M32599, nucleotides 590–754) was generously provided by Dr. Brian Liu. Plasmids were linearized, and antisense riboprobes were synthesized using T3 RNA polymerase

(GIBCO-BRL; Gaithersburg, MD). To compensate for the much greater abundance of the GAPDH transcript, ACE and GAPDH riboprobes were synthesized at different specific activities (3.92×10^8 and 7.38×10^7 counts $\cdot\text{min}^{-1}\cdot\mu\text{g}^{-1}$, respectively). The linearity of the assay was verified for both probes; 5×10^4 counts/min of each probe and 4.5 μg of total RNA were used in the RNase protection assay. RNA and diethyl pyrocarbonate-treated (DEPC) water were combined in a total volume of 8 μl , and 42 μl of buffer-containing probe were then added [final concentration: 72.24% formamide, 0.84 mM EDTA (pH 7.4), 0.3612 M NaCl, and 42 mM PIPES (pH 6.9)]. Samples were heat denatured at 85°C and then incubated overnight at 55°C . The samples were digested in a final concentration of 18 mM Tris (pH 7.4), 0.27 M NaCl, 4.54 mM EDTA (pH 7.4), 110 U/ml RNase T1, and 1.0 $\mu\text{g}/\text{ml}$ RNase A (RPA grade enzymes, Ambion). After RNase digestion, the samples were extracted with phenol-chloroform, ethanol precipitated, and resuspended in formamide loading dye [0.05% bromophenol blue, 0.05% xylene cyanol, 20 mM EDTA (pH 8.0), and 86% formamide]. The samples were separated on a $30 \times 40\text{-cm}$, 6% acrylamide-8 M urea gel. A radiolabeled DNA ladder (SeqMark, Research Genetics) was included for a size reference. After electrophoresis, the gel was dried and used to expose a Phosphorimager screen (Molecular Dynamics). The data were quantified using Quantity One software (Bio-Rad). Mouse kidney RNA from a single preparation was run in triplicate for each RNase protection assay and served as an interassay reference. This analysis was repeated at least twice for all tissues examined.

Western blot analysis. Membrane proteins were extracted from LV tissues using a modification of published methods (24). The pellet was resuspended with buffer (1:10 wt/vol; 20 mM NaPO₄, 0.5% SDS, and 3 M urea), briefly sonicated, and then clarified (4,340 g, 5 min). Membrane protein concentration was determined using the Bio-Rad Protein Assay kit II (6) using bovine serum albumin as the standard. The sample (20 μg) was electrophoresed using standard protocols (25), and gels were stained with Coomassie blue or transferred to 0.45- μm nitrocellulose membranes (38). Purified rabbit lung ACE (0.4 μg , Sigma; St. Louis, MO) served as a positive control. Anti-human ACE antibody (Peninsula Laboratories) was used at a concentration of 1 $\mu\text{g}/\text{ml}$, anti-actin antibody (clone C4, ICN Biomedicals) was used at a 1:3,000 dilution, and peroxidase-conjugated AffiniPure goat anti-mouse IgG (Jackson ImmunoResearch Laboratories) was used at 0.04 $\mu\text{g}/\text{ml}$. Bands were detected with chemiluminescence using Super Signal West Dura Extended Duration Substrate (Pierce). Band intensity was determined densitometrically using a Fluor-S Imager (Bio-Rad) and Quantity One software.

Statistical analysis. The data presented are representative of a minimum of two separate experiments. Data are expressed as means \pm SD. Differences between groups were assessed using an unpaired Student's *t*-test. In all cases, differences were considered significant when $P < 0.05$.

RESULTS

Gender differences in ventricular expression of murine ACE. To test whether gender differences exist in cardiac ACE expression in the adult mouse, a RNase protection assay was developed to measure ACE mRNA levels. A synthetic antisense RNA complementary to a 272-nucleotide region at the 5' end of the somatic isoform of murine ACE was produced. This segment of the ACE mRNA exhibits only 43% nucleo-

tide identity with the testis-specific isoform with the nucleotide identity restricted to stretches of 10 nucleotides or less. As a result, this riboprobe is specific to the somatic isoform of ACE. Quantitative detection of ACE transcript levels was assured by verifying that RNase protection was performed under conditions of probe excess.

ACE mRNA levels in 12-wk-old Swiss-Webster mice were measured by RNase protection and normalized to GAPDH levels to correct for variation in sample loading (Fig. 1A). Quantitation of the RNase protection assay revealed that ACE mRNA was significantly more abundant in ventricular RNA from male mice compared with female mice (Fig. 1, B and C; RV: 1.5-fold, $P < 0.05$; LV: 2.0-fold, $P < 0.0005$). This gender difference in ACE mRNA expression was seen in both the RV and LV (Fig. 1, B and C). It was not apparent in RNA isolated from the lung, whereas a slight gender difference was detected in the kidney (data not shown). Because ACE mRNA is substantially more abundant in the lung than in the heart, we questioned whether the lack of gender difference in lung ACE mRNA expression correlated with the robustness of ACE expression in that tissue. To address this, we repeated this analysis with total brain RNA. Despite similar ACE transcript abundance in brain and heart RNA, no gender difference in ACE expression was detected in the brain (Fig. 1D).

Because differences in RNA abundance do not always reflect differences in protein expression levels, we used Western blot analysis to determine whether gender differences exist in ventricular ACE protein levels. LV membrane proteins were extracted from 12-wk-old Swiss-Webster mice, and 20 μg of protein were immunoblotted using an anti-ACE antibody (11) with a preparation of purified rabbit lung ACE serving as a positive control. The lower one-half of the membrane was immunoblotted with an anti-actin antibody to provide a loading control (data not shown). A separate gel loaded with the identical samples was stained with Coomassie brilliant blue to verify sample integrity. Band intensity was measured using densitometry to demonstrate that ACE protein was 1.6 times ($P < 0.05$) more abundant in LV samples from male mice than from females (Fig. 2).

Because strain-dependent variations in cardiovascular parameters have been described in laboratory mice, we questioned whether a gender difference in cardiac ACE abundance was specific to the Swiss-Webster strain or whether it was apparent in other mouse strains as well. To address this question, we compared cardiac ACE mRNA levels in male and female mice from the C57/B16 strain using the RNase protection assay described above (Fig. 3). Male C57/B16 mice also had uniformly higher levels of ventricular ACE mRNA than age- and strain-matched females, although the

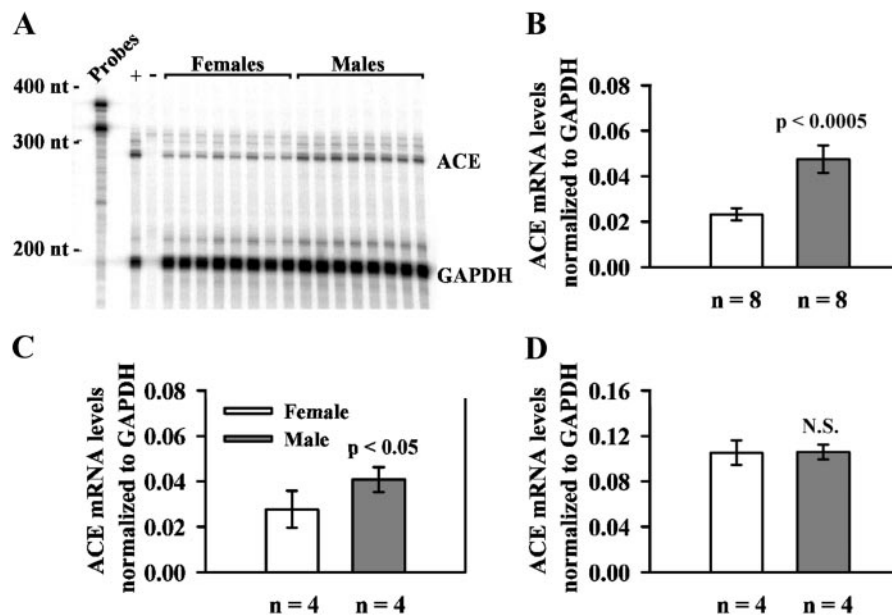


Fig. 1. Angiotensin-converting enzyme (ACE) mRNA levels are more abundant in left ventricular (LV) samples from male mice than from females. A: RNase protection assay was used to detect the transcripts for the somatic isoform of ACE [protected fragment: 272 nucleotides (nt)] and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; protected fragment: 164 nt) in LV RNA samples from 12-wk-old Swiss-Webster mice ($n = 8$ females and 8 males). Intact (undigested) probes for both molecules are included for comparison. Kidney RNA (+) and yeast tRNA (-) served as positive and negative controls, respectively. A representative experiment is shown. B-D: ACE mRNA levels in 12-wk-old Swiss-Webster mice were measured using an RNase protection assay and quantified using a phosphorimager. GAPDH mRNA levels measured in the same sample were used as a normalization factor to correct for variation in sample loading. ACE mRNA levels were significantly greater in ventricular RNA from male mice than from female mice with a slightly larger gender difference detected in LV (B) samples compared with right ventricular (RV; C) samples (2.0 times vs. 1.6 times). Bars represent means \pm SD. Despite relatively similar ACE transcript abundance in brain total RNA, no gender difference in ACE expression was detected in this tissue (D).

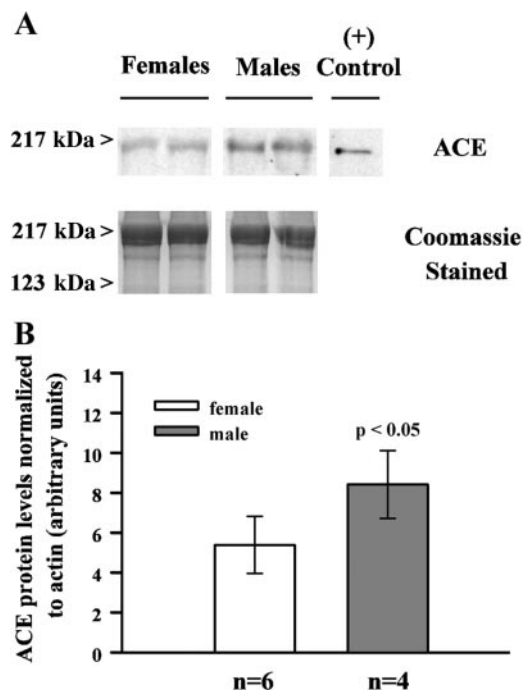


Fig. 2. Gender differences exist in LV ACE protein expression. LV membrane proteins were extracted from 12-wk-old Swiss-Webster mice, and 20 μ g of protein were immunoblotted using anti-ACE antibody. Purified rabbit lung ACE was used as a positive control, and a duplicate gel was stained with Coomassie brilliant blue to verify sample integrity. Only a small region of the Coomassie blue-stained gel is shown (A). The positions of the 217- and 123-kDa protein size markers are indicated. B: densitometry of the Western blots revealed that ACE protein levels were 1.6-fold greater ($P < 0.05$) in LV membrane preparations from male mice than from female mice. To adjust for possible variations in sample loading, the membranes were immunoblotted with an anti-actin antibody, and ACE immunoreactivity normalized to actin levels was also 1.6-fold greater in male mice ($n = 4$) than in female mice ($n = 6$). Bars represent means \pm SD.

difference between genders was smaller than that measured in the Swiss-Webster strain (Swiss-Webster: 2.0-fold, $P < 0.0005$; C57 B16: 1.4-fold, $P < 0.0005$). We also determined whether gender differences in cardiac ACE abundance were apparent in both young and old mice. No gender differences in ventricular ACE mRNA were seen in 6-wk-old C57/B16 mice (data not shown), but gender differences in cardiac ACE mRNA levels were apparent in 12-wk-old animals, and these differences persisted until at least 8 mo of age (Fig. 3).

Underlying gender differences in ACE expression result in greater absolute levels of ACE in male cardiomyopathic mice. Many investigators have shown that cardiac ACE levels increase in experimental models of cardiac hypertrophy or failure (4, 18, 26, 36) as well as in the failing human heart (28a). Are gender differences in ventricular ACE levels apparent in diseased hearts as well? To address this question, ventricular ACE mRNA levels were measured in a transgenic mouse model of cardiomyopathy (41) and compared with age- and gender-matched nontransgenic littermates. Ventricular ACE mRNA levels were increased in cardiomyopathic mice (Fig. 4, hatched bars) com-

pared with age- and sex-matched controls. Both male and female cardiomyopathic mice had increased levels of ventricular ACE relative to age- and sex-matched controls. However, when the absolute ACE mRNA levels were compared, it was clear that ACE mRNA levels were greatest in male cardiomyopathic animals (Fig. 4).

Surgical gonadectomy in male and female mice results in antithetical effects on ventricular ACE mRNA levels. Given the role of estrogen in influencing the levels of renin-angiotensin system components in non-cardiac tissues (see Refs. 7 and 30), we questioned whether gonadal steroids play a role in regulating the expression of the cardiac ACE levels in the mouse. To address this question, albeit indirectly, C57/B16 mice were subjected to surgical gonadectomy, RNA was prepared from the hearts of these animals, and left ventricular ACE mRNA levels were measured in surgically intact and gonadectomized mice. As expected, there was an increase in ventricular ACE mRNA levels in agonadal females (28% increase vs. surgically intact controls, $P < 0.05$). Unexpectedly, ventricular ACE mRNA levels in androgen-deprived males were substantially lower than in surgically intact males (37% decrease vs. surgically intact controls, $P < 0.0005$) and were equivalent to the levels measured in surgically intact females (Fig. 5).

DISCUSSION

We have presented data demonstrating a gender difference in the expression of ACE in the murine heart with greater cardiac ACE levels seen in male animals compared with females. Moreover, we have shown that ventricular ACE abundance is altered in agonadal animals and that male and female mice exhibit antithetical changes in ACE expression in response to surgical gonadectomy. It has been proposed that increased ACE abundance in the hypertrophied and failing heart may contribute to the local generation of angiotensin II and impact cardiac remodeling through local paracrine or autocrine effects (1, 31, 45). The greater abundance of ventricular ACE in males may contribute to the tendency of male rodents to develop cardiac dilation, which has been described in transgenic mouse models (23, 41), spontaneously hypertensive rats (43), and in response to LV pressure overload in rats (46) and in humans (9, 13, 42).

Potential mechanisms contributing to gender differences in cardiac ACE expression include negative regulation of ACE expression by estrogen and positive regulation of ACE expression by testosterone. Gallagher et al. (15) demonstrated that supraphysiological concentrations of estrogen in the rat (894 pg/ml) decrease ACE expression in the kidney, lung, and aorta (15). Our data are consistent with that report and suggest that estrogens also have a negative effect on the cardiac expression of ACE. No conserved estrogen response elements have been identified in 5' flanking DNA from ACE genes (19, 20, 47). However, there is a putative activator protein-1 (AP-1) site located \sim 300

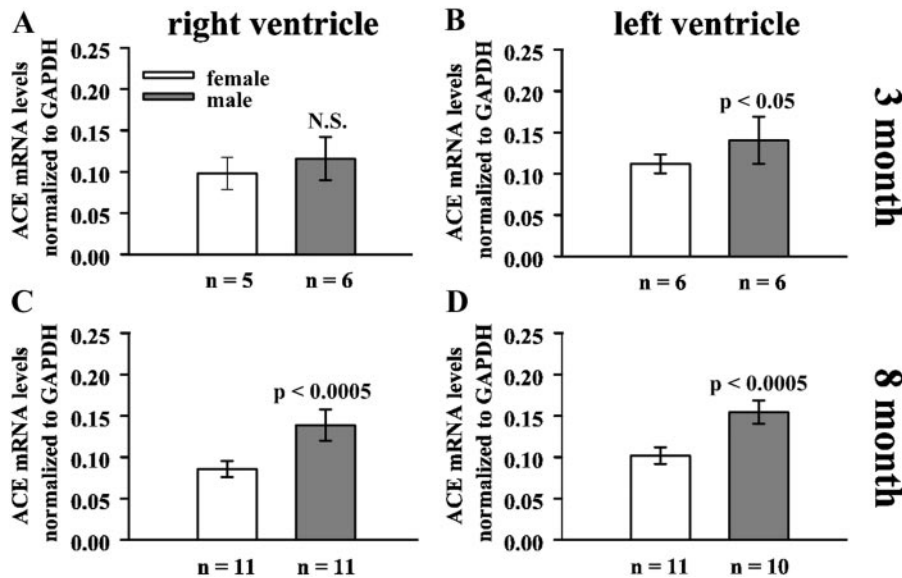


Fig. 3. Gender differences in ventricular ACE mRNA levels are also seen in C57/Bl6 mice, and these differences persist with age. LV ACE mRNA levels were measured in C57/Bl6 mice using a RNase protection assay. Ventricular ACE mRNA levels were greater in male C57/Bl6 mice than in female mice. This gender difference was apparent in both 3- (A and B) and 8-mo-old animals (C and D), but in C57/Bl6 mice the difference in the RV only reached statistical significance in the older animals. Bars represent means \pm SD. NS, not significant.

bp 5' to the transcriptional start site in human, rabbit, and mouse ACE genes, and AP-1 sites have been shown to be negatively responsive to estrogen in other genes. In addition, because ventricular ACE mRNA levels were decreased in androgen-deprived males, we suggest that testosterone may play a role in regulating ACE expression in the mouse heart. However, further experimentation is needed to determine whether ligand-bound steroid hormone receptors regulate ACE expression directly.

Although the testis-specific ACE isoform is positively regulated by androgens (40), studies on the regulation of the somatic ACE isoform by nonestrogenic steroids have focussed on the effects of glucocorticoids. Dexamethasone treatment increases ACE activity in cultured endothelial cells (12) and cultured cardiac fibroblasts (5, 17). However, Dasarathy et al. (12) reported that testosterone did not stimulate and estrogens did not decrease ACE activity in cultured bovine pulmonary artery endothelial cells (12). It is not clear whether the lack of stimulation of ACE by testosterone described by Dasarathy et al. (12) in cultured endothe-

lial cells reflects the response of all ACE-expressing cells in vivo. A number of cell types have been shown to express ACE, including endothelial cells, vascular smooth muscle cells, and activated macrophages. However, it is not clear whether cells other than endothelial and vascular smooth muscle cells express ACE in the healthy adult mouse heart. We also do not know whether the gender difference in cardiac ACE expression we report in this study reflects differential ACE expression in several cardiac cell types or differential expression in a subset of cells. Furthermore, gender differences in ACE expression were not detected in all tissues. Because we expect that different tissues within an individual mouse would be exposed to the same hormonal milieu, this suggests that the hormonal milieu is necessary but not sufficient to generate a gender difference in ACE expression. In addition, no correlation was seen between the robustness of ACE expres-

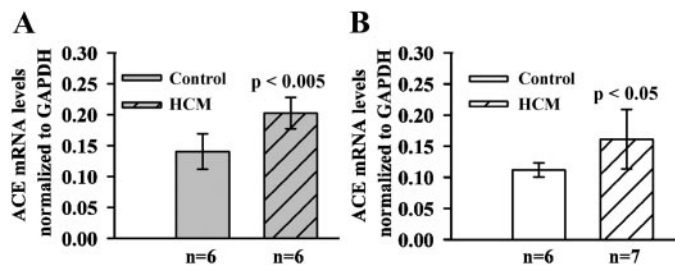


Fig. 4. Absolute levels of LV ACE mRNA are greater in the LV of male compared with female cardiomyopathic mice (HCM). Ventricular ACE mRNA levels were measured in transgenic cardiomyopathic mice and compared with age- and gender-matched nontransgenic littermates. Ventricular ACE mRNA levels were increased in cardiomyopathic mice compared with age- and sex-matched controls, and the absolute levels of ACE mRNA levels were greater in male animals (A) than in females (B). Bars represent means \pm SD.

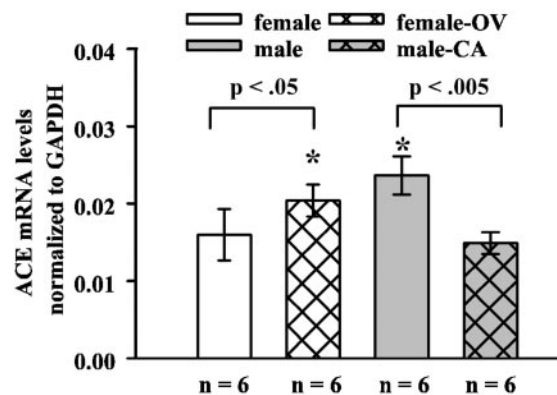


Fig. 5. Surgical gonadectomy in male and female mice results in altered ventricular ACE mRNA levels. LV ACE mRNA levels were measured in surgically intact and gonadectomized animals. Antithetical changes in LV ACE mRNA levels were seen in the gonadectomized male and female mice. Bars represent means \pm SD. OV, ovariectomized females; CA, castrated males. * P < 0.05 versus all other groups.

sion in a tissue and the presence of a gender difference in its expression. For steroid hormone receptors to influence the expression of the ACE gene, the appropriate hormone receptors must be coexpressed in the target cell. ACE expression in different tissues also may be influenced by the presence of tissue-specific coactivators, such as FLH2, a cardiac-specific coactivator of the androgen receptor (28a). To clarify whether this scenario operates in the heart, further studies are needed to identify cells in the male and female mouse heart that coexpress ACE, the androgen receptor, or estrogen receptors (α or β) and coactivators for these steroid-hormone receptors.

A number of investigators have demonstrated increased cardiac ACE levels in failing hearts. It is interesting to note that the gender difference in cardiac ACE levels that we report in healthy mice (1.4-fold in C57/Bl6 mice and 2.0-fold in Swiss-Webster mice) are similar in magnitude to the differences reported between healthy and failing hearts of several species (for examples, see Refs. 4, 18, 36, and 49). Moreover, we demonstrated that in a cardiomyopathic mouse model, ventricular ACE mRNA levels were increased in both male and female animals, but the absolute levels of ACE were greatest in hearts from male cardiomyopathic mice. The functional significance of this difference remains to be determined. However, if the heart is taking up angiotensin I from the circulation, these gender differences in cardiac ACE expression could prove sufficient to influence local production of angiotensin II. On the basis of our findings, we propose that cardiac production of angiotensin II is greater in the hearts of male mice than female mice and that this increased local capacity to generate angiotensin II is sufficient to influence the remodeling of the heart when a pathological stimulus is imposed. Further studies are needed to determine whether these gender differences in tissue ACE expression exist in humans and to clarify the roles of estrogens and testosterone in regulating these differences.

REFERENCES

1. **Bader M, Peters J, Baltatu O, Muller DN, Luft FC, and Ganten D.** Tissue renin-angiotensin systems: new insights from experimental animal models in hypertension research. *J Mol Med* 79: 76–102, 2001.
2. **Bai S, Campbell SE, Moore JA, Morales MC, and Gerdes AM.** Influence of age, growth, and sex on cardiac myocyte size and number in rats. *Anat Rec* 226: 207–212, 1990.
3. **Baker PJ, Ramey ER, and Ramwell PW.** Androgen-mediated sex differences of cardiovascular responses in rats. *Am J Physiol Heart Circ Physiol* 235: H242–H246, 1978.
4. **Barlucchi L, Leri A, Dostal DE, Fiordaliso F, Tada H, Hintze TH, Kajstura J, Nadal-Ginard B, and Anversa P.** Canine ventricular myocytes possess a renin-angiotensin system that is upregulated with heart failure. *Circ Res* 88: 298–304, 2001.
5. **Barreto-Chaves MLM, Aneas I, and Krieger JE.** Glucocorticoid regulation of angiotensin-converting enzyme in primary culture of adult cardiac fibroblasts. *Am J Physiol Regul Integr Comp Physiol* 280: R25–R32, 2001.
6. **Bradford M.** A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248–254, 1976.
7. **Brosnihan KB, Senanayake PS, Li P, and Ferrario CM.** Bi-directional actions of estrogen on the renin-angiotensin system. *Braz J Med Biol Res* 32: 373–381, 1999.
8. **Camper-Kirby D, Welch S, Walker A, Shiraishi I, Satchell KR, Schaefer E, Kajstura J, Anversa P, and Sussman MA.** Myocardial Akt activation and gender-increased nuclear activity in females versus males. *Circ Res* 88: 1020–1027, 2001.
9. **Carroll JD, Carroll EP, Feldman T, Ward DM, Lang RM, McGaughey D, and Karp RB.** Sex-associated differences in left ventricular function in aortic stenosis of the elderly. *Circulation* 86: 1099–1107, 1992.
10. **Chomczynski P and Sacchi N.** Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162: 156–169, 1987.
11. **Danilov S, Jaspard E, Churakova T, Towbin H, Savoie F, Wei L, and Alhenc-Gelas F.** Structure-function analysis of angiotensin I-converting enzyme using monoclonal antibodies. Selective inhibition of the amino-terminal active site. *J Biol Chem* 269: 26806–26814, 1994.
12. **Dasarathy Y, Lanzillo JJ, and Fanburg BL.** Stimulation of bovine pulmonary artery endothelial cell ACE by dexamethasone: involvement of steroid receptors. *Am J Physiol Lung Cell Mol Physiol* 263: L645–L649, 1992.
13. **Douglas PS, Katz SE, Weinberg EO, Chen MH, Bishop SP, and Lorell BH.** Hypertrophic remodeling: gender differences in the early response to left ventricular pressure overload. *J Am Coll Cardiol* 32: 1118–1125, 1998.
14. **Foegh ML, Asotra S, Howell MH, and Ramwell PW.** Estradiol inhibition of arterial neointimal hyperplasia after balloon injury. *J Vasc Surg* 19: 722–726, 1994.
15. **Gallagher PE, Li P, Lenhart JR, Chappell MC, and Brosnihan KB.** Estrogen regulation of angiotensin-converting enzyme mRNA. *Hypertension* 33: 323–328, 1999.
16. **Gray GA, Sharif I, Webb DJ, and Seckl JR.** Oestrogen and the cardiovascular system: the good, the bad and the puzzling. *Trends Pharmacol Sci* 22: 152–156, 2001.
17. **Hafizi S, Wharton J, Morgan K, Allen SP, Chester AH, Catravas JD, Polak JM, and Yacoub MH.** Expression of functional angiotensin-converting enzyme and AT₁ receptors in cultured human cardiac fibroblasts. *Circulation* 98: 2553–2559, 1998.
18. **Hirsch AT, Talsness CE, Schunkert H, Paul M, and Dzau VJ.** Tissue-specific activation of cardiac angiotensin converting enzyme in experimental heart failure. *Circ Res* 69: 475–482, 1991.
19. **Howard HL, McLaughlin-Taylor E, and Hill RL.** The effect of mouse euthanasia technique on subsequent lymphocyte proliferation and cell mediated lympholysis assays. *Lab Anim Sci* 40: 510–514, 1990.
20. **Hubert C, Houot AM, Corvol P, and Soubrier F.** Structure of the angiotensin I-converting enzyme gene. Two alternate promoters correspond to evolutionary steps of a duplicated gene. *J Biol Chem* 266: 15377–15383, 1991.
21. **Hulley S, Grady D, Bush T, Furberg C, Herrington D, Riggs B, and Vittinghoff E.** Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/Progestin Replacement Study (HERS) Research Group. *JAMA* 280: 605–613, 1998.
22. **Jun SS, Chen Z, Pace MC, and Shaul PW.** Estrogen upregulates cyclooxygenase-1 gene expression in ovine fetal pulmonary artery endothelium. *J Clin Invest* 102: 176–183, 1998.
23. **Kadokami T, McTiernan CF, Kubota T, Frye CS, and Feldman AM.** Sex-related survival differences in murine cardiomyopathy are associated with differences in TNF-receptor expression. *J Clin Invest* 106: 589–597, 2000.
24. **Kinoshita A, Urata H, Bumpus FM, and Husain A.** Measurement of angiotensin I converting enzyme inhibition in the heart. *Circ Res* 73: 51–60, 1993.
25. **Laemmli UK.** Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680–685, 1970.
26. **Lee YA, Liang CS, Lee MA, and Lindpaintner K.** Local stress, not systemic factors, regulate gene expression of the cardiac renin-angiotensin system in vivo: a comprehensive study

- of all its components in the dog. *Proc Natl Acad Sci USA* 93: 11035–11040, 1996.
27. **Mendelsohn ME and Karas RH.** The protective effects of estrogen on the cardiovascular system. *N Engl J Med* 340: 1801–1811, 1999.
 28. **Moore LG, McMurtry IF, and Reeves JT.** Effects of sex hormones on cardiovascular and hematologic responses to chronic hypoxia in rats. *Proc Soc Exp Biol Med* 158: 658–664, 1978.
 - 28a. **Muller JM, Isele U, Metzger E, Rempel A, Moser M, Pscherer A, Breyer T, Holubarsch C, Buettner R, and Schule R.** FHL2, a novel tissue-specific coactivator of the androgen receptor. *EMBO J* 19: 359–369, 2000.
 29. **Nuedling S, Kahlert S, Loebbert K, Doevendans PA, Meyer R, Vetter H, and Grohe C.** 17 β -Estradiol stimulates expression of endothelial and inducible NO synthase in rat myocardium in-vitro and in-vivo. *Cardiovasc Res* 43: 666–674, 1999.
 30. **Oelkers WK.** Effects of estrogens and progestogens on the renin-aldosterone system and blood pressure. *Steroids* 61: 166–171, 1996.
 31. **Pratt RE.** Angiotensin II and the control of cardiovascular structure. *J Am Soc Nephrol* 10: S120–S128, 1999.
 32. **Reckelhoff JF.** Gender differences in the regulation of blood pressure. *Hypertension* 37: 1199–1208, 2001.
 33. **Schiabale TF, Penpargkul S, and Scheuer J.** Differences in male and female rats in cardiac conditioning. *J Appl Physiol* 50: 112–117, 1981.
 34. **Schiabale TF and Scheuer J.** Effects of physical training by running or swimming on ventricular performance of rat hearts. *J Appl Physiol* 46: 854–860, 1979.
 35. **Schaible TF and Scheuer J.** Comparison of heart function in male and female rats. *Basic Res Cardiol* 79: 402–412, 1984.
 36. **Schunkert H, Dzau VJ, Tang SS, Hirsch AT, Apstein CS, and Lorell BH.** Increased rat cardiac angiotensin converting enzyme activity and mRNA expression in pressure overload left ventricular hypertrophy. Effects on coronary resistance, contractility and relaxation. *J Clin Invest* 86: 1913–1920, 1990.
 37. **Sharkey LC, Holycross BJ, Park S, Shiry LJ, Hoepf TM, McCune SA, and Radin MJ.** Effect of ovariectomy and estrogen replacement on cardiovascular disease in heart failure-prone SHHF/Mcc-fa cp rats. *J Mol Cell Cardiol* 31: 1527–1537, 1999.
 38. **Towbin M, Staehelin T, and Gordon J.** Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedures and some applications. *Proc Natl Acad Sci USA* 76: 4350–4354, 1979.
 39. **van Eickels M, Grohe C, Cleutjens JP, Janssen BJ, Wellens HJ, and Doevendans PA.** 17 β -Estradiol attenuates the development of pressure-overload hypertrophy. *Circulation* 104: 1419–1423, 2001.
 40. **Velletri PA, Aquilano DR, Bruckwick E, Tsai-Morris CH, Dufau ML, and Lovenberg W.** Endocrinological control and cellular localization of rat testicular angiotensin-converting enzyme (EC 3.4.151). *Endocrinology* 116: 2516–2522, 1985.
 41. **Vikstrom KL, Factor SM, and Leinwand LA.** Mice expressing mutant myosin are a model for hypertrophic cardiomyopathy. *Mol Med* 2: 556–567, 1996.
 42. **Villarreal FJ and Dillmann WH.** Cardiac hypertrophy-induced changes in mRNA levels for TGF- β ₁, fibronectin, and collagen. *Am J Physiol Heart Circ Physiol* 262: H1861–H1866, 1992.
 43. **Wallen WJ, Cserti C, Belanger MP, and Wittnich C.** Gender-differences in myocardial adaptation to afterload in normotensive and hypertensive rats. *Hypertension* 36: 774–779, 2000.
 44. **Wassmann S, Baumer AT, Strehlow K, van Eickels M, Grohe C, Ahlborn K, Rosen R, Bohm M, and Nickenig G.** Endothelial dysfunction and oxidative stress during estrogen deficiency in spontaneously hypertensive rats. *Circulation* 103: 435–441, 2001.
 45. **Weber KT.** Extracellular matrix remodeling in heart failure. A role for de novo angiotensin II generation. *Circulation* 96: 4065–4082, 1997.
 46. **Weinberg EO, Thienelt CD, Katz SE, Bartunek J, Tajima M, Rohrbach S, Douglas PS, and Lorell BH.** Gender differences in molecular remodeling in pressure overload hypertrophy. *J Am Coll Cardiol* 34: 264–273, 1999.
 47. **Xavier-Neto J, Pereira AC, Junqueira ML, Carmona R, and Krieger JE.** Rat angiotensin-converting enzyme promoter regulation by beta-adrenergics and cAMP in endothelium. *Hypertension* 34: 31–38, 1999.
 49. **Zisman LS, Asano K, Dutcher DL, Ferdensi A, Robertson AD, Jenkin M, Bush EW, Bohlmeier T, Perryman MB, and Bristow MR.** Differential regulation of cardiac angiotensin converting enzyme binding sites and AT₁ receptor density in the failing human heart. *Circulation* 98: 1735–1741, 1998.